

Figure 1A

[illegible]

Figure 1B

| | | | | | | | | | | |
|-----|-----------|-------------|------------|-------------|--------------|------------|------------|------------|-------------|-----|
| 401 | M4cDNA | TGATTGGGAA | AAAAACCGAC | AAGCATTAGC | CAAAATGCGC | CAAGTTTGTG | CAAAGGGAAC | AACTGGCGGA | TTGGGAGGGG | 480 |
| | M6cDNA | TGATTGGGAA | AAAAACCGAC | AAGCATTAGC | CAAAATGCGC | CAAGTTTGTG | CAAAGGGAAC | AACTGGCGGA | TTGGGAGGGG | |
| | M8cDNA | TGATTGGGAA | AAAAACCGAC | AAGCATTAGC | CAAAATGCGC | CAAGTTTGTG | CAAAGGGAAC | AACTGGCGGA | TTGGGAGGGG | |
| | M15cDNA | TGATTGGGAA | AAAAACCGAC | AAGCATTAGC | CAAAATGCGC | CAAGTTTGTG | CAAAGGGAAC | AACTGGCGGA | TTGGGAGGGG | |
| | Consensus | TGATTGGGAA | AAAAACCGAC | AAGCATTAGC | CAAAATGCGC | CAAGTTTGTG | CAAAGGGAAC | AACTGGCGGA | TTGGGAGGGG | |
| 481 | M4cDNA | AAATCTACGT | GGTGACTGAT | TGTTTCAGATG | ACAAATGCTGC | AAATCCAAAG | CCAGGGACAC | TTCGTTGTGG | TGTCACCCCAA | 560 |
| | M6cDNA | AAATCTACGT | GGTGACTGAT | TGTTTCAGATG | ACAAATGCTGC | AAATCCAAAG | CCAGGGACAC | TTCGTTGTGG | TGTCACCCCAA | |
| | M8cDNA | AAATCTACGT | GGTGACTGAT | TGTTTCAGATG | ACAAATGCTGC | AAATCCAAAG | CCAGGGACAC | TTCGTTGTGG | TGTCACCCCAA | |
| | M15cDNA | AAATCTACGT | GGTGACTGAT | TGTTTCAGATG | ACAAATGCTGC | AAATCCAAAG | CCAGGGACAC | TTCGTTGTGG | TGTCACCCCAA | |
| | Consensus | AAATCTACGT | GGTGACTGAT | TGTTTCAGATG | ACAAATGCTGC | AAATCCAAAG | CCAGGGACAC | TTCGTTGTGG | TGTCACCCCAA | |
| 561 | M4cDNA | GATAAACCTT | TGTGGATCAT | CTTTAAGAAA | GATATGGTCA | TAAACCTTAA | ACACGAGCTT | GTGATAAACA | AAGACAAGAC | 640 |
| | M6cDNA | GATAAACCTT | TGTGGATCAT | CTTTAAGAAA | GATATGGTCA | TAAACCTTAA | ACACGAGCTT | GTGATAAACA | AAGACAAGAC | |
| | M8cDNA | GATAAACCTT | TGTGGATCAT | CTTTAAGAAA | GATATGGTCA | TAAACCTTAA | ACACGAGCTT | GTGATAAACA | AAGACAAGAC | |
| | M15cDNA | GATAAACCTT | TGTGGATCAT | CTTTAAGAAA | GATATGGTCA | TAAACCTTAA | ACACGAGCTT | GTGATAAACA | AAGACAAGAC | |
| | Consensus | GATAAACCTT | TGTGGATCAT | CTTTAAGAAA | GATATGGTCA | TAAACCTTAA | ACACGAGCTT | GTGATAAACA | AAGACAAGAC | |
| 641 | M4cDNA | AATTGATGGA | AGAGGTGCAA | ATGTTGAGAT | CACCTTGTTGGC | GGTCTCACCA | TTCACAACGT | TTGCAATGTG | ATCATTTCATA | 720 |
| | M6cDNA | AATTGATGGA | AGAGGTGCAA | ATGTTGAGAT | CACCTTGTTGGC | GGTCTCACCA | TTCACAACGT | TTGCAATGTG | ATCATTTCATA | |
| | M8cDNA | AATTGATGGA | AGAGGTGCAA | ATGTTGAGAT | CACCTTGTTGGC | GGTCTCACCA | TTCACAACGT | TTGCAATGTG | ATCATTTCATA | |
| | M15cDNA | AATTGATGGA | AGAGGTGCAA | ATGTTGAGAT | CACCTTGTTGGC | GGTCTCACCA | TTCACAACGT | TTGCAATGTG | ATCATTTCATA | |
| | Consensus | AATTGATGGA | AGAGGTGCAA | ATGTTGAGAT | CACCTTGTTGGC | GGTCTCACCA | TTCACAACGT | TTGCAATGTG | ATCATTTCATA | |
| 721 | M4cDNA | ACATTTCACAT | ACATGATATT | AAAGTAACCG | AAGGTGGAAT | TATTAAGGCA | ACGGACGCTA | AACCAGGACA | TAGACATAAG | 800 |
| | M6cDNA | ACATTTCACAT | ACATGATATT | AAAGTAACCG | AAGGTGGAAT | TATTAAGGCA | ACGGACGCTA | AACCAGGACA | TAGACATAAG | |
| | M8cDNA | ACATTTCACAT | ACATGATATT | AAAGTAACCG | AAGGTGGAAT | TATTAAGGCA | ACGGACGCTA | AACCAGGACA | TAGACATAAG | |
| | M15cDNA | ACATTTCACAT | ACATGATATT | AAAGTAACCG | AAGGTGGAAT | TATTAAGGCA | ACGGACGCTA | AACCAGGACA | TAGACATAAG | |
| | Consensus | ACATTTCACAT | ACATGATATT | AAAGTAACCG | AAGGTGGAAT | TATTAAGGCA | ACGGACGCTA | AACCAGGACA | TAGACATAAG | |

Figure 1c

| | | | | | | | | | | |
|------|-----------|------------|------------|------------|------------|-------------|------------|-------------|------------|------|
| 801 | M4cDNA | AGCGACGGAG | ATGGTATTGG | TGTTGCTGGT | TCTTCAAAGA | TATGGATCGA | TCATTGCACA | CTTAGTCAATG | GTCCAGATGG | 880 |
| | M6cDNA | AGCGACGGAG | ATGGTATTGG | TGTTGCTGGT | TCTTCAAAGA | TATGGATCGA | TCATTGCACA | CTTAGTCAATG | GTCCAGATGG | |
| | M8cDNA | AGCGACGGAG | ATGGTATTGG | TGTTGCTGGT | TCTTCAAAGA | TATGGATCGA | TCATTGCACA | CTTAGTCAATG | GTCCAGATGG | |
| | M15cDNA | AGCGACGGAG | ATGGTATTGG | TGTTGCTGGT | TCTTCAAAGA | TATGGATCGA | TCATTGCACA | CTTAGTCAATG | GTCCAGATGG | |
| | Consensus | AGCGACGGAG | ATGGTATTGG | TGTTGCTGGT | TCTTCAAAGA | TATGGATCGA | TCATTGCACA | CTTAGTCAATG | GTCCAGATGG | |
| 881 | M4cDNA | CCTTATTGAT | GTACCGTTGG | GTAGCACAGC | CGTTACCAAT | TCCAATTGCA | AATTTAGCCA | TCACCAAAAA | ATTCTATTAC | 960 |
| | M6cDNA | CCTTATTGAT | GTACCGTTGG | GTAGCACAGC | CGTTACCAAT | TCCAATTGCA | AATTTAGCCA | TCACCAAAAA | ATTCTATTAC | |
| | M8cDNA | CCTTATTGAT | GTACCGTTGG | GTAGCACAGC | CGTTACCAAT | TCCAATTGCA | AATTTAGCCA | TCACCAAAAA | ATTCTATTAC | |
| | M15cDNA | CCTTATTGAT | GTACCGTTGG | GTAGCACAGC | CGTTACCAAT | TCCAATTGCA | AATTTAGCCA | TCACCAAAAA | ATTCTATTAC | |
| | Consensus | CCTTATTGAT | GTACCGTTGG | GTAGCACAGC | CGTTACCAAT | TCCAATTGCA | AATTTAGCCA | TCACCAAAAA | ATTCTATTAC | |
| 961 | M4cDNA | TCGGAGCAGA | CAATTCACAT | GTAGACGATA | AAAAAATGCA | TGTCACAGTA | GCATTCAACA | GGTTGCGAGA | AGCATGTGAT | 1040 |
| | M6cDNA | TCGGAGCAGA | CAATTCACAT | GTAGACGATA | AAAAAATGCA | TGTCACAGTA | GCATTCAACA | GGTTGCGAGA | AGCATGTGAT | |
| | M8cDNA | TCGGAGCAGA | CAATTCACAT | GTAGACGATA | AAAAAATGCA | TGTCACAGTA | GCATTCAACA | GGTTGCGAGA | AGCATGTGAT | |
| | M15cDNA | TCGGAGCAGA | CAATTCACAT | GTAGACGATA | AAAAAATGCA | TGTCACAGTA | GCATTCAACA | GGTTGCGAGA | AGCATGTGAT | |
| | Consensus | TCGGAGCAGA | CAATTCACAT | GTAGACGATA | AAAAAATGCA | TGTCACAGTA | GCATTCAACA | GGTTGCGAGA | AGCATGTGAT | |
| 1041 | M4cDNA | CAAGAATGC | CACGATGTCG | ATTGGATT | TTCCAAGTTG | TTAACAAATGA | CTACACCAGC | TGGGGAACGT | ACGCCATTGG | 1120 |
| | M6cDNA | CAAGAATGC | CACGATGTCG | ATTGGATT | TTCCAAGTTG | TTAACAAATGA | CTACACCAGC | TGGGGAACGT | ACGCCATTGG | |
| | M8cDNA | CAAGAATGC | CACGATGTCG | ATTGGATT | TTCCAAGTTG | TTAACAAATGA | CTACACCAGC | TGGGGAACGT | ACGCCATTGG | |
| | M15cDNA | CAAGAATGC | CACGATGTCG | ATTGGATT | TTCCAAGTTG | TTAACAAATGA | CTACACCAGC | TGGGGAACGT | ACGCCATTGG | |
| | Consensus | CAAGAATGC | CACGATGTCG | ATTGGATT | TTCCAAGTTG | TTAACAAATGA | CTACACCAGC | TGGGGAACGT | ACGCCATTGG | |
| 1121 | M4cDNA | TGGTAGTGCC | AATCCTACTA | TCCTTAGCCA | AGGCAACCGA | TTCCATGCTC | CGAATGACCC | AATGAAGAAA | AATGTGTTGG | 1200 |
| | M6cDNA | TGGTAGTGCC | AATCCTACTA | TCCTTAGCCA | AGGCAACCGA | TTCCATGCTC | CGAATGACCC | AATGAAGAAA | AATGTGTTGG | |
| | M8cDNA | TGGTAGTGCC | AATCCTACTA | TCCTTAGCCA | AGGCAACCGA | TTCCATGCTC | CGAATGACCC | AATGAAGAAA | AATGTGTTGG | |
| | M15cDNA | TGGTAGTGCC | AATCCTACTA | TCCTTAGCCA | AGGCAACCGA | TTCCATGCTC | CGAATGACCC | AATGAAGAAA | AATGTGTTGG | |
| | Consensus | TGGTAGTGCC | AATCCTACTA | TCCTTAGCCA | AGGCAACCGA | TTCCATGCTC | CGAATGACCC | AATGAAGAAA | AATGTGTTGG | |



Figure 1D

| | |
|-----------|---|
| 1201 | 1280 |
| M4cDNA | TGAGGGCTGA TGCACCATAT ACAGAGTCAA TGAAGTGGAA TTGGAGATCT GAGAAAGACT TGTTAGAAAA TGGAGCTATA |
| M6cDNA | TGAGGGCTGA TGCACCATAT ACAGAGTCAA TGAAGTGGAA TTGGAGATCT GAGAAAGACT TGTTAGAAAA TGGAGCTATA |
| M8cDNA | TGAGGGCTGA TGCACCATAT ACAGAGTCAA TGAAGTGGAA TTGGAGATCT GAGAAAGACT TGTTAGAAAA TGGAGCTATA |
| M15cDNA | TGAGGGCTGA TGCACCATAT ACAGAGTCAA TGAAGTGGAA TTGGAGATCT GAGAAAGACT TGTTAGAAAA TGGAGCTATA |
| Consensus | TGAGGGCTGA TGCACCATAT ACAGAGTCAA TGAAGTGGAA TTGGAGATCT GAGAAAGACT TGTTAGAAAA TGGAGCTATA |
| 1281 | 1360 |
| M4cDNA | TTTGTAGCAT CAGGGTGCGA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA TTTGATTCCA GCTGAACCAG GATCAGCAGT |
| M6cDNA | TTTGTAGCAT CAGGGTGCGA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA TTTGATTCCA GCTGAACCAG GATCAGCAGT |
| M8cDNA | TTTGTAGCAT CAGGGTGCGA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA TTTAATTCCA GCTGAACCAG GATCAGCAGT |
| M15cDNA | TTTGTAGCAT CAGGGTGCGA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA TTTGATTCCA GCTGAACCAG GATCAGCAGT |
| Consensus | TTTGTAGCAT CAGGGTGCGA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA TTTGATTCCA GCTGAACCAG GATCAGCAGT |
| 1361 | 1440 |
| M4cDNA | TCTTCAACTC ACCAGTTGTG CTGGCACGCT CAAATGCGTT CCTGGAAAAC CTGTGTAATA GTTATCACCC ACTTTTATT |
| M6cDNA | TCTTCAACTC ACCAGTTGTG CTGGCACGCT CAAATGCGTT CCTGGAAAAC CTGTGTAATA GTTATCACCC ACTTTTATT |
| M8cDNA | TCTTCAACTC ACCAGTTGTG CTGGCACGCT CAAATGCGTT CCTGGAAAAC CTGTGTAATA GTTATCACCC ACTTTTATT |
| M15cDNA | TCTTCAACTC ACCAGTTGTG CTGGCACGCT CAAATGCGTT CCTGGAAAAC CTGTGTAATA GTTATCACCC ACTTTTATT |
| Consensus | TCTTCAACTC ACCAGTTGTG CTGGCACGCT CAAATGCGTT CCTGGAAAAC CTGTGTAATA GTTATCACCC ACTTTTATT |
| 1441 | 1520 |
| M4cDNA | TTTATTGTTA TTAGCATTTT TTATACTTGT TAGGATTGTA GTGGAATGAG ACATTGATAC GTGCATTACA AGACCATTC |
| M6cDNA | TTTATTGTTA TTAGCATTTT TTATACTTGT TAGGATTGTA GTGGAATGAG ACATTGATAC GTGCATTACA AGACCATTC |
| M8cDNA | TTTATTGTTA TTAGCATTTT TTATACTTGT TAGGATTGTA GTGGAATGAG ACATTGATAC GTGCATTACA AGACCAAAAA |
| M15cDNA | TTTATTGTTA TTAGCATTTT TTATACTTGT TAGGATTGTA GTGGAATGAG ACATTGATAC GTGCATTACA AGACCAAAAA |
| Consensus | TTTATTGTTA TTAGCATTTT TTATACTTGT TAGGATTGTA GTGGAATGAG ACATTGATAC GTGCATTACA AGACCATTC |
| 1521 | 1570 |
| M4cDNA | TCAACATATT TTGCTACTAT CACATGTTCA CGTTAAAAA AAAAAA |
| M6cDNA | TCAACATATT TTGCTAAAA- -----AAAAA AAAAAA |
| M8cDNA | AAAAA AAAAAA |
| M15cDNA | AAAAA AAAAAA |
| Consensus | tCAACAtAtt ttgcta..a.aaaaa aaaaaaa |

Figure 2

cDNA and amino acid sequences of M4, M6, M15 (identical clones):

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atggaaaaacattatTTTgttatattgttcaccgcagcgtttgttttcgtgggtgcagct
M E K H Y F V I L F T A A F V F V G A A
gctcgggctgacattggtgatgagctcgaagcggctcaatttaattcaacaaggaggggc
A R A D I G D E L E A A Q F N S T R R G
ttacacgaatgtgcagcacataacataatagacaagtgttgagggtgcaaagctgattgg
L H E C A A H N I I D K C W R C K A D W
gaaaaaaaccgacaagcattagccaaatgcgcgcaagggttttgcaaagggaacaactggc
E K N R Q A L A K C A Q G F A K G T T G
ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaatcca
G L G G E I Y V V T D C S D D N A A N P
aagccagggacacttcgttgtggtgtcaccgaagataaacctttgtggatcatctttaag
K P G T L R C G V T Q D K P L W I I F K
aaagatatggtcataaaacttaaacacgagcttgtgataaacaagacaagacaattgat
K D M V I K L K H E L V I N K D K T I D
ggaagaggtgcaaattgttgagatcacttgtggcgggtctcaccattcacaacgtttgcaat
G R G A N V E I T C G G L T I H N V C N
gtgatcattcataacattcacatacatgatattaaagtaaccgaagggtggaattattaag
V I I H N I H I H D I K V T E G G I I K
gcaacggacgctaaaccaggacatagacataagagcgacggagatggtatttgtgttgct
A T D A K P G H R H K S D G D G I C V A
ggttcttcaaagatatggatcgatcattgcacacttagtcatggtccagatggccttatt
G S S K I W I D H C T L S H G P D G L I
gatgtcacgttgggtagcacagccgttaccattttccaattgcaaatttagccatcaccaa
D V T L G S T A V T I S N C K F S H H Q
aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca
K I L L L G A D N S H V D D K K M H V T
gtagcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga
V A F N R F A E A C D Q R M P R C R F G
tttttccaagttgttaacaatgactacaccagctggggaacgtacgccattggtggtagt
F F Q V V N N D Y T S W G T Y A I G G S
gccaatcctactatccttagccaaggcaaccgattccatgctccgaatgacccaatgaag
A N P T I L S Q G N R F H A P N D P M K
aaaaatgtgttggtgagggctgatgcaccacatacagagtcaatgaagtggaattggaga
K N V L V R A D A P H T E S M K W N W R
tctgagaaagacttgttagaaaatggagctatatTTTgtagcatcaggggtgcgacccgcat
S E K D L L E N G A I F V A S G C D P H
ctaaccccggaaacaaaaaagccatttgattccagctgaaccaggatcagcagttcttcaa
L T P E Q K S H L I P A E P G S A V L Q
ctcaccagttgtgctggcagcgtcaaatgcgttcctggaaaaccttgtaa
L T S C A G T L K C V P G K P C -
```

Figure 3**cDNA and amino acid sequence of clone M8:**

atggaaaaacattatTTTTgttatattgttcaccgcagcgtttgttttcgtgggtgcagct
M E K H Y F V I L F T A A F V F V G A A
gctcgggctgacattgggtgatgagctcgaagcggctcaatttaattcaacaaggaggggc
A R A D I G D E L E A A Q F N S T R R G
ttacacgaatgtgcagcacataacataatagacaagtgttgagggtgcaaagctgattgg
L H E C A A H N I I D K C W R C K A D W
gaaaaaaaccgacaagcattagccaaatgcgcgcaagggttttgcaaagggaacaactggc
E K N R Q A L A K C A Q G F A K G T T G
ggattgggaggggaaatctacgtgggtgactgattgttcagatgacaatgctgcaaattcca
G L G G E I Y V V T D C S D D N A A N P
aagccaggggacacttcgttgtggtgtcacccaagataaacctttgtggatcatcttcaag
K P G T L R C G V T Q D K P L W I I F K
aaagatatggtcataaaacttaaacacgagcttgtgataaacaagacaagacaattgat
K D M V I K L K H E L V I N K D K T I D
ggaagaggtgcaaattgttgagatcacttgtggcgggtctcaccattcacaacgtttgcaat
G R G A N V E I T C G G L T I H N V C N
gtgatcattcataacattcacatacatgatattaaagtaacggaagggtggaattattaag
V I I H N I H I H D I K V T E G G I I K
gcaacgggacgctaaaccagggcatagacataagagcgacggagatggattttgtgttgct
A T D A K P G H R H K S D G D G I C V A
ggttcttcgaagatatggatcgatcattgcacacttagtcattgggtccagatggccttatt
G S S K I W I D H C T L S H G P D G L I
gatgtcacgttgggtagcacagccgttaccattttccaattgcaaatttagccatcaccaa
D V T L G S T A V T I S N C K F S H H Q
aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca
K I L L L G A D N S H V D D K K M H V T
gtcgcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga
V A F N R F A E A C D Q R M P R C R F G
tttttccaagttgttaacaatgactacaccagctggggaacgtacgccattgggtggtagc
F F Q V V N N D Y T S W G T Y A I G G S
gccaatcctactatccttagccaaggcaaccgattccatgctcccaatgacccaatgaag
A N P T I L S Q G N R F H A P N D P M K
aaaaatgtgttggtgagggtgatgcaccacatacagagtcaatgaagtgggaattggaga
K N V L V R A D A P H T E S M K W N W R
tctgagaaagacttgttagaaaaatggagctatatattgttagcatcaggggtgcgacccgc
S E K D L L E N G A I F V A S G C D P H
ctaaccgggaacaaaaagccatttaattccagctgaaccaggatcagcagttcttcaa
L T P E Q K S H L I P A E P G S A V L Q
ctcaccagttgtgctggcagcgtcaaattgcgttcctggaaaaccttgtaa
L T S C A G T L K C V P G K P C -

Figure 4

pHIS-Parallel2

T7 Promoter-> Lac Operator Xba I
 GAAATTAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTTAACTTTAAGAA

Nde I
 GGAGATATACAT ATG TCG TAC TAC CAT CAC CAT CAC CAT CAC GAT TAC GAT ATC CCA ACG ACC
 Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro Thr Thr
 6xHis Spacer Region

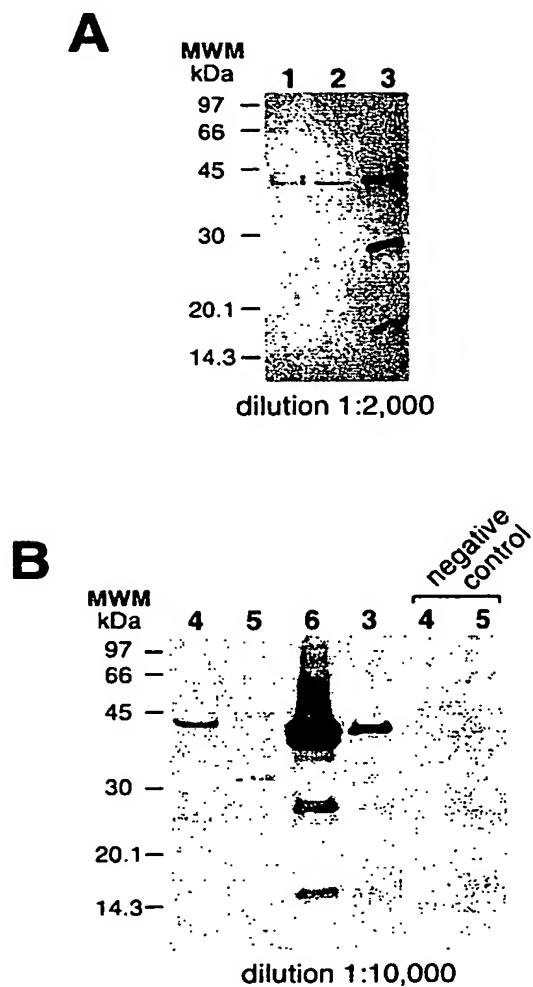
Ehe I Nco I Bam HI Eco RI Stu I Sal I Sst I
 GAA AAC CTG TAT TTT CAG GGC GCC ATG GGA TCC GGA ATT CAA AGG CCT ACG TCG ACG AGC
 Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser
 rTEV Protease
 Cleavage Site

Spe I Not I Nsp V Xba I Pst I Xho I
 TCA ACT AGT GCG GCC GCT TTC GAA TCT AGA GCC TGC AGT CTC GAG CAC CAC CAC CAC CAC
 Ser Thr Ser Ala Ala Ala Phe Glu Ser Arg Ala Cys Ser Leu Glu His His His His His

CAC TGA GAT CCG GCT GCT AAC AAA GCC CGA AAG GAA GCT GAG TTG GCT GCT GCC ACC GCT
 His ***

*Non-unique sites

Figure 5

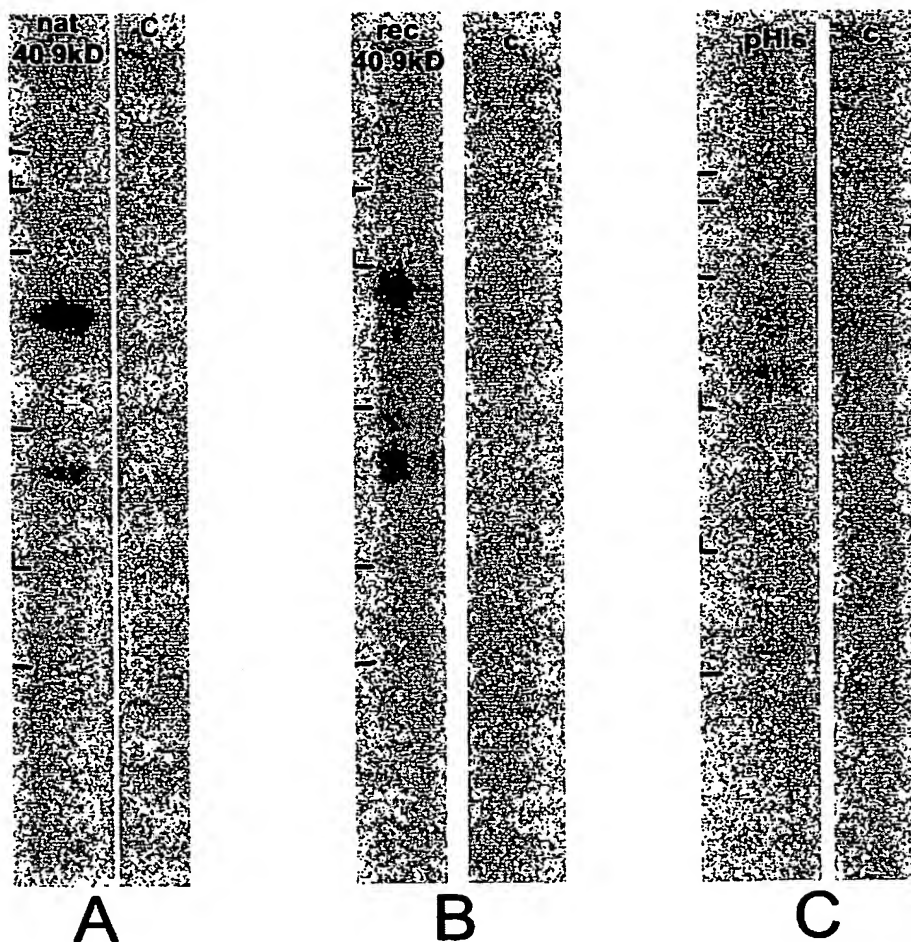
Immunoblot with rabbit anti-Amb a 1 antibodies

- 1 - Mugwort pollen extract
- 2 - Purified mugwort pollen allergen
- 3 - Purified Amb a 1 from ragweed pollen (natural Amb a 1)
- 4 - Recombinant mugwort allergen
- 5 - Control bacterial lysate
- 6 - Ragweed pollen extract

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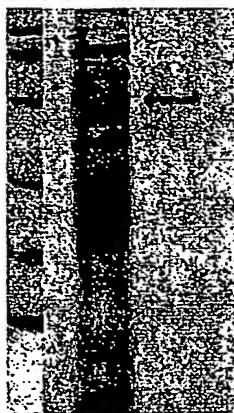
Figure 6

IgE blot with NIH patient



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Figure 7



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